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Drug release from α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide-based microparticles

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Abstract

Spherical pH-sensitive microparticles have been prepared by reverse phase suspension polymerization technique. Starting polymer has been α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide (PHEA) partially derivatized with glycidylmethacrylate (GMA). PHEA-GMA copolymer (PHG) has been crosslinked in the presence of acrylic acid (AA) or methacrylic acid (MA) at various concentration. The obtained microparticles have been characterized by FT-IR spectrophotometry, particle size distribution analysis and scanning electron microscopy. In order to have information about water affinity of the prepared samples, swelling measurements have been carried out in aqueous media which simulate some biological fluids. The possibility to employ the prepared samples as pH-sensitive microparticles has been investigated by performing in vitro release studies. Experimental data have showed that the release rate from these microparticles depends on the environmental pH and the chemical structure of the drug.

Keywords: αβ-poly(*N*-2-hydroxyethyl)-DL-aspartamide; Glycidylmethacrylate; Radical crosslinking; pH-sensitive hydrogels; Drug release

1. Introduction

A considerable number of strategies have been developed in order to obtain drug delivery systems (DDSs) for an effective therapy. In this contest, hydrogels have attracted considerable attention due to their inertness and good biocompatibility [1–3].

Their capacity to entrap drugs and, successively, to release them in an aqueous medium and the possibility to regulate such release by controlling their swelling in physiological fluids make hydrogels excellent candidates for the controlled release of pharmaceuticals [4,5].

It is possible to prepare hydrogels shaped as microparticles by using different polymerization techniques [6,7]. Among these, reverse-phase suspension polymerization method allows to obtain spherical microparticles with a narrow size distribution [8].

Recently, stimuli-responsive hydrogels have attracted the attention of many research groups since they are able to undergo an abrupt volume change owing to small changes of environmental conditions, such as pH [9,10], temperature, ionic strength, electric and magnetic fields [11–14]. In particular, pH is the most widely utilized signal to regulate drug release from hydrogels after oral administration.

Even if several macromolecules have been successfully utilized to prepare pH-sentivive hydrogels [15–18], the research of new biocompatible polymers suitable to prepare these systems represents an interesting incentive for several researchers. In particular, polyaminoacids have received a great interest because of their protein-like nature and the possibility of preparation by synthesis. Being similar to natural proteins, polyaminoacids can be considered as a compromise between natural and synthetic macromolecules; these polymers can be planned so that they have a suitable molecular weight, biodegradability or not and appropriate functional groups.

In previous papers [19,20] we have reported the preparation of hydrogels via radical reaction employing as starting material the copolymer PHEA-GMA (PHG) obtained by partial derivatization of a polyaminoacid, such as α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide (PHEA) [21] with glycidylmethacrylate (GMA) [22].

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The functionalization of PHEA with GMA allowed to introduce reactive vinyl groups in the side chain in order to facilitate radical reactions and to obtain biodegradable hydrogels, due to the presence of esters bonds in the network structure.

Aim of the present study has been the preparation and characterization of a new family of microparticles having a polyaminoacidic structure and able to show a different swelling behaviour as a function of the environmental pH. For this reason, PHG has been crosslinked through a reverse phase suspension polymerization technique in the presence of acidic monomers such as acrylic or methacrylic acid (AA or MA, respectively).

The synthesis has been carried by using various amounts of acidic monomer, in order to obtain microparticles with different physicochemical properties, such as the swelling behaviour as a function of the environmental pH. The sample that has showed the greater swelling in the simulated intestinal fluid, has been loaded with various drugs. In vitro release studies in simulated gastrointestinal fluids have showed the influence of the environmental pH and the chemical nature of entrapped drug on release profile.

2. Experimental

2.1. Apparatus

Molecular weights of starting PHEA and PHG copolymer were determined by light scattering measurements, using a Dawn DSP-F Laser Spectra Physics Spectrometer.

^{1}H-NMR spectra were obtained with a Bruker AC-250 instrument operating at 250.13 MHz. Samples were solubilized in D_2O.

FT-IR spectra were recorded as pellets in KBr in the range 4000–400 cm^{-1} using a Perkin–Elmer 1720 Fourier Transform Spectrophotometer. The resolution was 1 cm^{-1}. The number of scans was 100.

Particle size distribution and aqueous dynamic swelling measurements were carried out using an image processor and analysis system Leica Quantimet Q 500 equipped with a Leica Wild 3D stereomicroscope. This image processor calculates the particle area and converts it to equivalent circle diameter.

High-pressure liquid chromatography (HPLC) analyses were carried out using a Varian 9012 liquid chromatograph equipped with a Rheodyne 7125 injector (fitted with a 10 μl loop), a Kontron HPLC 432 detector and a Hewlett-Packard 3394 integrator. For the analyses a reversed-phase C_{18} column (μ Bondapak; 10 μm of 250 × 4.6 mm internal diameter, obtained from Waters) was used.

The scanning electron microscopy (SEM) photographs were obtained with a Leo stereoscan 420; the sample surface was made conductive by the deposition of a layer of gold in a vacuum chamber.

X-ray diffraction analysis was performed using a diffractometer Philips PW 1729 X-ray generator. The experimental parameters were: Cu$K\alpha$ radiation, tube setting 40 KV, 20 mA; angular speed 2° (2θ/min); range recorded 10–40° (2θ/min); time costant 1 s, chart speed 2 cm/min.

2.2. Materials

All the reagents used were of analytical grade, unless otherwise stated. Acrylic acid (AA) and methacrylic acid (MA) were distilled before using.

Anhydrous *N,N*-dimethylacetamide (DMA), *n*-hexane, carbon tetrachloride and 2-(4-imidazolyl)-ethylamine >97% (histamine base) were provided by Fluka Chemie.

(S)(+)4-isobutyl-alpha-methylphenyl-acetic acid 99% (ibuprofen), 4-(2-aminoethyl)phenol 99% (tyramine), acrylic acid 99%, methacrylic acid 99%, glycidyl methacrylate (GMA), 4-dimethylaminopyridine (4-DMAP) 99.9%, sorbitan trioleate (Span 85), *N,N,N′,N′*-tetramethylethylenediamine (TMEDA) and ammonium persulfate were purchased from Aldrich Chemical Co.

5-(2,4-Difluorophenyl)salicylic acid (diflunisal) and 2,4-diamino-5-(3,4,5-trimethoxybenzyl)-pyrimidine (trimethoprim) were purchased from Sigma.

Dexamethasone was kindly supplied by S.I.F.I. (Catania–Italy).

α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide (PHEA) was prepared according to a procedure elsewhere reported [21]. The batch of PHEA used in the present study had a weight-average molecular weight of 56,900 ($M_w/M_n = 1.79$).

Derivatization of PHEA with GMA to obtain PHG copolymer was carried out in organic phase (anhydrous DMA), using 4-DMAP as catalyst, purified and characterized according to a procedure elsewhere reported [22]. The degree of derivatization (DD) of prepared PHG, determined by ^{1}H-NMR resulted to be 28 ± 1 mol%. The weight-average molecular weight of PHG copolymer determined by light scattering measurements, was 61,000 ($M_w/M_n = 1.86$).

2.3. Preparation of microparticles

In a typical experiment, a mixture of *n*-hexane (20 ml) and carbon tetrachloride (18 ml) was placed in a cylindrical glass reaction vessel fitted with an anchor-type stirrer and thermostated at 35°C. After 30 min N_2 bubbling, this mixture was treated with 3 ml of distilled water containing PHG (350 mg), acidic monomer (AA)

or (MA) (at various concentration) and 80 mg of ammonium persulfate.

The density of the organic phase was adjusted by the addition of *n*-hexane or carbon tetrachloride so that the aqueous phase sank slowly when stirring stopped. With the stirrer at 1000 rpm, the mixture was treated with 150 μl of Span 85, then after 10 min with 150 μl of TMEDA and the stirring was continued for 3 h.

The microparticles so obtained were filtered, washed with 100 ml portions of 2-propanol, ethanol and acetone. These samples have been reported in the text as:

PHG-AA (2.27), PHG-AA (2.77), PHG-AA (3.65), PHG-MA (2.27), PHG-MA (2.77), PHG-MA (3.65) and PHG-MA (5.48). The number reported in brackets indicates the molar ratio of AA or MA to PHG repeating unit.

Microparticles of PHG alone were also prepared using a procedure elsewhere reported [19] and they have been reported in the text as PHG (a).

2.4. Drug loading by soaking procedure

PHG-MA (5.48) microparticles were soaked, for 3 days at room temperature and under magnetic stirring, in a concentrated drug solution. Dexamethasone, ibuprofen and diflunisal were solubilized in ethanol, histamine in water, tyramine in hot ethanol and trimethoprim in methanol/water (3/2). The amount of drug solubilized was chosen in order to have a drug loading of 20% (w/w). The solvent was removed by evaporation under reduced pressure and the microparticles were dried at 10^{-1} mmHg in the presence of P_2O_5 until constant weight. The same procedure was employed to soak the sample PHG (a) with ibuprofen, dexamethasone and histamine.

2.5. Determination of water regain percentage

Aliquots (80–100 mg) of the prepared microparticles dried to constant weight, by heating to 60°C/0.01 Torr, were placed in a tared 5 ml sintered glass filter (∅ 10 mm; porosity, G3), weighed, and left to swell by immersing the filter plus support in a beaker containing the swelling media, i.e. doubly distilled water, HCl 0.1 N (pH 1, simulated gastric fluid) or phosphate buffer (NaCl, Na_2HPO_4, KH_2PO_4) pH 6.8 (simulated intestinal fluid). After 24 h, the excess liquid was removed by percolation at atmospheric pressure. Then the filter was placed in a properly sized centrifuge test tube by fixing it with the help of a bored silicone stopper, then centrifuged at 350 times gravity acceleration for 15 min and weighed. The filter tare was determinated after centrifugation with water alone. Water regain percentage was calculated by the following equation:

$$\text{Water regain percentage (WR\%)} = (W_s - W_d)/W_d \times 100$$

being W_s and W_d the weights of the swollen and initial dry microparticles, respectively.

Each experiment was carried out in triplicate and the results were in agreement within ±4% standard error.

2.6. Measurements of dynamic swelling

Aqueous dynamic swelling was determined by observing through an optical stereomicroscope equipped with an image processor (see the apparatus) the variation of microparticle diameter in the swelling media, i.e. doubly distilled water, HCl 0.1 N (pH 1, simulated gastric fluid)) or phosphate buffer (NaCl, Na_2HPO_4, KH_2PO_4) pH 6.8 (simulated intestinal fluid), at room temperature until the microparticles achieved the full swollen equilibrium with a diameter d_∞. The values of normalized diameter, d_t/d_0 were determined, being d_t the diameter of swollen microparticle at time t and d_0 the diameter of initial dry microparticle.

The experiment was carried by analysing twenty microparticles of each samples and the results were in agreement within ±3% standard error.

2.7. Drug stability at pH 1.0 and 6.8

The stability of each drug was studied at pH 1.0 and 6.8. Aliquots of drug (10 mg) were incubated at 37°C in HCl 0.1 N (pH 1) or phosphate buffer (NaCl, Na_2HPO_4, KH_2PO_4) pH 6.8 solution. At scheduled time intervals, samples were withdrawn and assayed by HPLC, in order to determine the drug concentration.

HPLC conditions were:

- for dexamethasone: ammonium acetate 35.5 mM (pH 5.5 corrected by adding acetic acid)/acetonitrile (7/3), 1.0 ml/min flow, UV detection at $\lambda = 242$ nm;
- for diflunisal: H_3PO_4 (0.1% v/v)/methanol (5/95), 1.0 ml/min flow, UV detection at $\lambda = 254$ nm;
- for histamine and tyramine: KH_2PO_4 20 mM (pH 3 corrected by adding 85% H_3PO_4), 0.5 ml/min flow, UV detection at $\lambda = 215$ nm;
- for ibuprofen: acetic acid (5 g/l)/acetonitrile (1/1), 1.0 ml/min flow, UV detection at $\lambda = 254$ nm;
- for trimethoprim: $NaClO_4$ 11 mM (pH 3.1 corrected by adding 85% H_3PO_4)/methanol (7/3), 1.3 ml/min flow, UV detection at $\lambda = 280$ nm.

2.8. Drug release at pH 1.0 and 6.8 from microparticles

Aliquots (10 mg) of drug-loaded PHG-MA (5.48) microparticles were dispersed in flasks containing HCl 0.1 N (pH 1.0, simulated gastric fluid) and maintained at 37 ± 0.1°C in a water bath for 2 h with magnetic stirring

(100 rpm). After this time, a solution of 0.2 M tribasic sodium phosphate was added to raise the pH to 6.8 (simulated intestinal fluid), according to the method reported in USP XXII (drug-release test, method A for enteric-coated particles). Sink conditions were maintained throughout the experiment. Then, at suitable time intervals, samples were filtered and analysed by HPLC.

Each experiment was carried out in triplicate and the results were in agreement within ±5% standard error.

3. Results and discussion

Aqueous solutions of PHG have been crosslinked in the presence of acrylic acid (AA) or methacrylic acid (MA) by radical reaction through a reverse phase suspension polymerization technique schematized in Fig. 1.

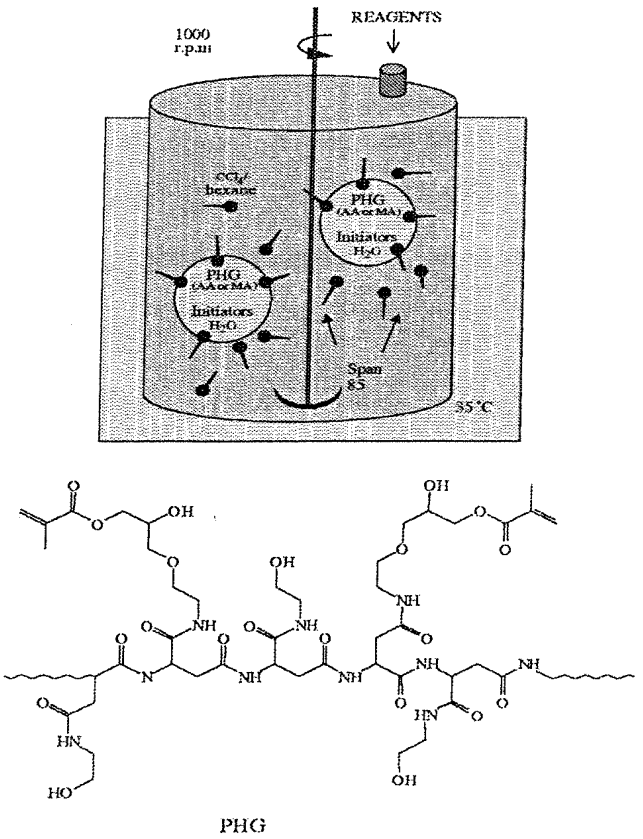

Fig. 1. Schematic representation of the reverse phase suspension polymerization technique employed to crosslink PHG in the presence of acrylic (AA) or methacrylic acid (MA) and chemical structure of PHG.

In our experiments we have prepared PHG-AA and PHG-MA crosslinked samples by using various molar ratios (X) between acidic monomer (AA or MA) and PHG repeating unit as reported in Table 1. It is evident that, for the reaction between PHG and MA, it is possible to obtain microparticles by using an X value until 5.48, whereas the same amount does not allow to obtain spherical microparticles for PHG-AA. In fact, for PHG-AA, values of X greater than 3.65 cause the formation of non-spherical and large aggregates.

N,N,N',N'-tetramethylethylenediamine (TMEDA) and ammonium persulfate have been employed to initiate the formation of methacrylic (belonging to PHG and MA) and acrylic (belonging to AA) radicals. It can be supposed that the chains of PHG are connected by some hydrocarbon bridges responsible for the network formation, whereas acrylic and methacrylic acid are randomly linked to PHG chains as acidic pendant groups.

The FT-IR analysis of all obtained samples shows the disappearance of bands at 1405 and 951 cm^{-1}, awardable to PHG methacrylic groups and at 934 and 949 cm^{-1} awardable to vinyl group of acrylic and methacrylic acid, respectively. This suggests that the reaction proceeds by opening of the double bonds of all reagents.

Considering that the employed technique allows to obtain spherical microparticles, we have evaluated the effect of the nature and concentration of the acidic monomer on microparticle size. In particular, we have determined the value of equivalent circle diameter of microparticles by using a stereomicroscope equipped with an image processor (see the apparatus).

Fig. 2, reports as an example, the comparison between the dimensional profiles of PHG-AA and PHG-MA prepared with the same X value (2.77). It is evident an asymmetric but narrow size distribution and no significant difference in the value of maximum equivalent diameter for PHG-AA and PHG-MA when the same X value is employed. On the contrary, for each series, an increase of the maximum equivalent diameter is observed by increasing the X value (see Fig. 3).

The image processor calculates also the roundness index which has been always near 1 for all PHG-AA and PHG-MA samples. This index is a parameter that gives

Table 1
Values of molar ratio (X) between acidic monomer (AA or MA) and PHG repeating unit employed to prepare PHG-AA and PHG-MA microparticles

Sample	PHG-AA PHG-MA	PHG-AA PHG-MA	PHG-AA PHG-MA	PHG-MA
X	2.27	2.77	3.65	5.48

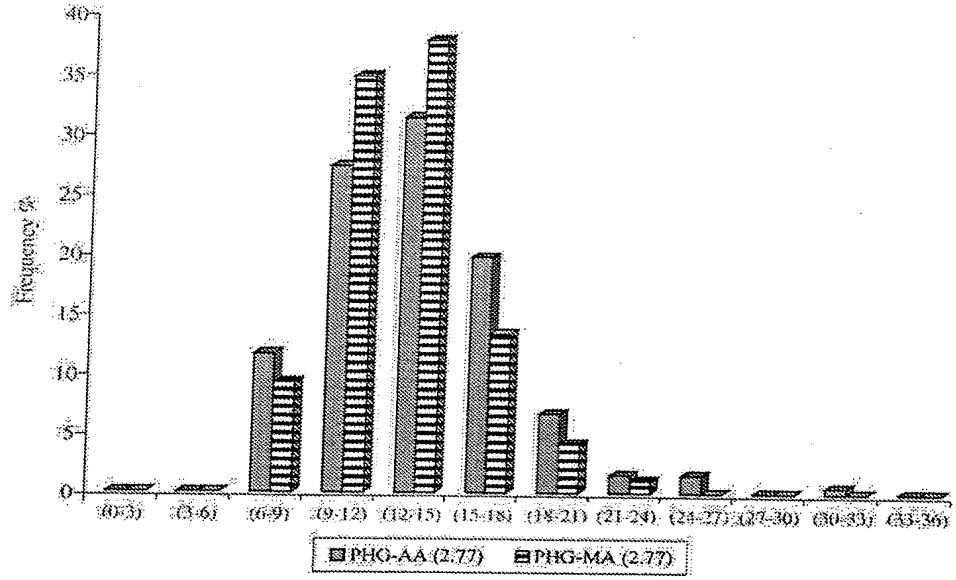

Fig. 2. Size distribution profiles of PHG-AA (2.77) and PHG-MA (2.77) samples. The number reported in brackets indicates the molar ratio between AA or MA and PHG repeating unit.

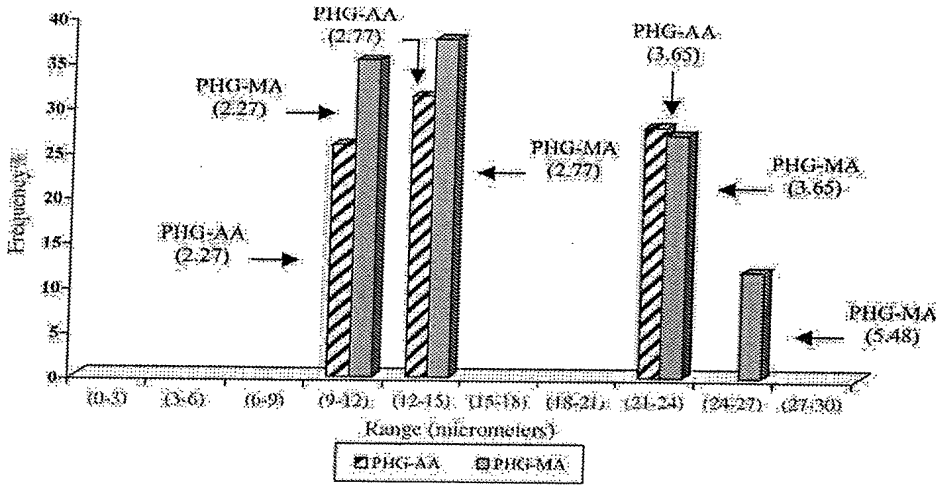

Fig. 3. Frequency % versus maximum equivalent diameter of all PHG-AA and PHG-MA samples. The number reported in brackets indicates the molar ratio between AA or MA and PHG repeating unit.

information about the particle shape. It has been calculated by the following ratio: $(\text{Perimeter})^2/(4\pi \cdot \text{Area} \cdot 1.064)$ where 1.064 is a correction factor for the angles produced by the image digitalization. Values of roundness index corresponding to 1 indicate a spherical shape.

The results of dimensional analysis have been also confirmed through scanning electron microscopy (SEM). As an example we report in Fig. 4 the SEM micrographs of PHG-MA (5.48) that reveal the spherical shape of the sample, the rather narrow dimensional distribution and its porous outside surface, according to the formation of a crosslinked structure. Similar results have been obtained for the other samples. The shape and the morphology of the prepared microparticles suggest their potential use as drug delivery systems. In fact, the spherical shape allows to eliminate the anisotropic swelling normally associated with others geometries, the presence of micropores could facilitate the drug release and the rather homogeneous size distribution could determine a uniform drug delivery from the microparticles.

In addition, the presence of pendant acidic groups (AA or MA) suggests the potential application of these microparticles as pH-sensitive systems. Therefore, in order to evaluate their affinity towards the aqueous

medium and their behaviour as a function of environmental pH, the values of water regain percentage (WR %) have been determined in doubly distilled water as well as in simulated gastric (pH 1.0) and intestinal (pH 6.8) juices, chosen as representative fluids of the gastrointestinal tract. In Table 2 we report the values of WR % in various media and the ratio between the swelling at pH 6.8 and pH 1 (Y) for PHG-AA and PHG-MA microparticles.

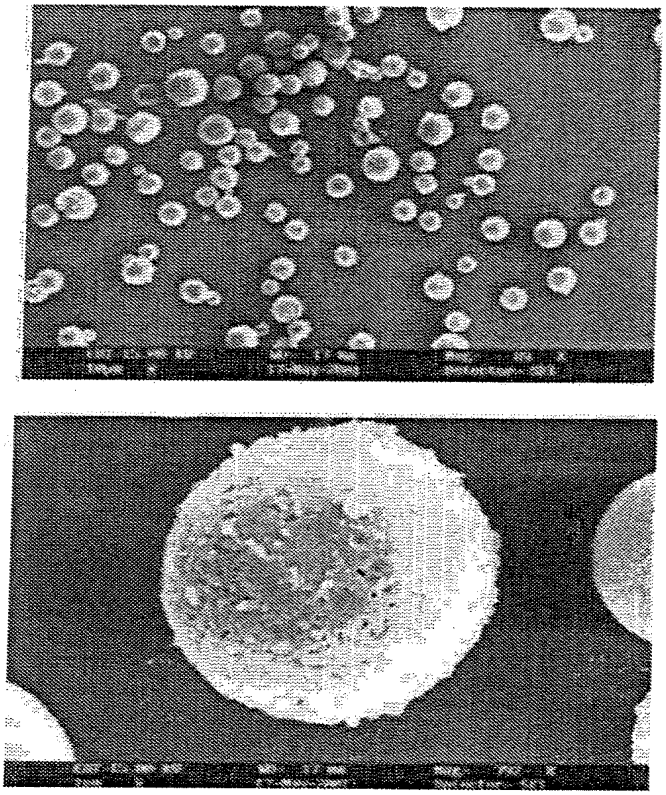

Fig. 4. SEM micrographs of the sample PHG-MA (5.48). The number reported in brackets indicates the molar ratio between MA and PHG repeating unit.

The reported data evidence a different swelling behaviour as a function of the pH of the penetrant medium. In particular, as expected, at pH 1.0 there is a considerable lowering of the WR % due to the presence of pendant acidic groups, undissociated at this value of pH. For each series, at pH 1, the WR % value decreases by increasing the amount of acidic monomer; the minimum value has been obtained for the sample PHG-MA (5.48) prepared with the maximum amount of methacrylic acid.

When the pH is 6.8, the water regain is greater than that found at pH 1 and the WR % value increases by increasing the amount of acidic monomer as a consequence of electrostatic repulsions between polymeric chains due to the increase of dissociated groups at this pH value. However the values of WR % at pH 6.8 are lower than those found in doubly distilled water due to the ionic strength and the osmotic pressure of the first medium. These data have been compared with those of PHG crosslinked in the absence of acidic monomers (sample PHG (a)). For this sample the swelling decreases also in the order doubly distilled water > pH 6.8 > pH 1.0 (data reported in Ref. [19]) but for the Y value is only 1.2, i.e. significantly lower of Y values found for PHG-AA and PHG-MA samples.

Analogous results have been obtained by performing dynamic swelling measurements. In particular, we have evaluated the variation of microparticle diameter as a function of time, when these samples are immersed in various penetrant media.

Fig. 5 reports, as an example, the values of normalized diameter (d_t/d_0) as a function of time of the samples PHG-MA (5.48) and PHG (a) in various media such as doubly distilled water, HCl 0.1 N (pH 1) and phosphate buffer (pH 6.8) solution.

As it can be observed, the normalized diameter of microparticles of both samples increases monotonically towards the equilibrium swollen value (d_∞), following the same trend discussed for WR % values, i.e. it decreases in the order doubly distilled water > pH 6.8 > pH 1.0. Analogous behaviour has been found for

Table 2
Values of water regain % (WR %) and ratio between the swelling at pH 6.8 and 1 (Y) of PHG-AA and PHG-MA microparticles in various media (values are means ± standard error ($n = 3$))

Sample	WR %			Y (pH 6.8/pH 1.0)
	H_2O	pH 6.8	pH 1	
PHG-AA (2.27)[a]	310 ± 3	270 ± 2	134 ± 3	2.0
PHG-AA (2.77)	320 ± 4	281 ± 2	120 ± 2	2.3
PHG-AA (3.65)	358 ± 7	298 ± 3	109 ± 3	2.7
PHG-MA (2.27)	456 ± 4	316 ± 4	182 ± 4	1.7
PHG-MA (2.77)	669 ± 1	330 ± 1	170 ± 1	1.9
PHG-MA (3.65)	674 ± 2	414 ± 2	154 ± 4	2.7
PHG-MA (5.48)	681 ± 3	482 ± 1	77 ± 2	6.3

[a]The number reported in brackets indicates the molar ratio between AA or MA and PHG repeating unit.

the other prepared samples. It is also evident that for the PHG (a) sample, the difference between the normalized diameter values at pH 1 and 6.8 is lower that that found for PHG-MA (5.48) sample.

All these results demonstrate that the presence of acidic pendant groups in PHG network cause a different swelling as a function of environmental pH. This allows supposing that the prepared microparticles are able to release physically entrapped drugs owing to pH variations of a physiological medium.

To confirm this assumption, the sample that has showed the greatest difference in the swelling between pH 1.0 and 6.8, i.e. the sample PHG-MA (5.48), has been loaded with various drugs containing or not functional groups ionizable as a function of the environmental pH. In particular the entrapped drugs were: ibuprofen (pKa 4.4) and diflunisal (pKa 2.9), having carboxylic groups, histamine (pKa 10.1), tyramine (pKa 9.7–10.7) [23] and trimethoprim (pKa 7.3), having amine groups, and dexamethasone, as an example of neutral drug in aqueous media.

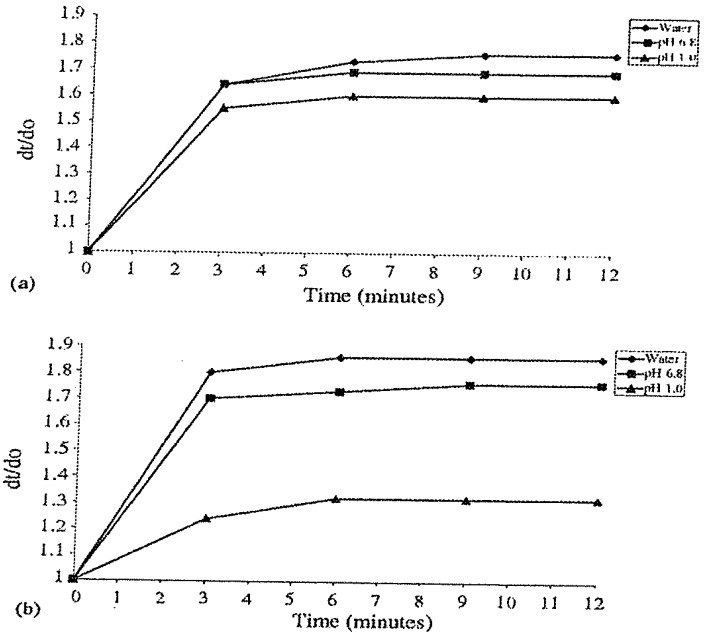

Fig. 5. Normalized diameter values (d_t/d_o) versus time for (a) PHG (a) and (b) PHG-MA (5.48) samples in different swelling media. The number reported in brackets indicates the molar ratio between MA and PHG repeating unit.

The determination of the drug dispersion state in PHG-MA (5.48) sample was performed by X-ray analysis. Fig. 6 reports, as an example, the X-ray diffraction patterns of pure diflunisal, unloaded and diflunisal loaded PHG-MA (5.48) samples. It is evident that pure diflunisal is in the crystalline state; on the contrary, both the drug unloaded and loaded PHG-MA (5.48) microparticles are in the amorphous state. The obtained results demonstrate that during the polymerization/crosslinking reaction no crystalline region was formed and that the drug is molecularly entrapped inside the network, i.e. in a physical state readily available to the dissolution process in a release medium. Analogous results have been found for PHG-MA (5.48) microparticles containing the others chosen drugs.

In order to have information about the processes that occur during the swelling of drug containing microparticles, we have performed dynamic swelling measurements of these samples in doubly distilled water, phosphate buffer pH 6.8 and HCl solution pH 1.0 (see Fig. 7).

Drug containing microparticles swell until the diameter reaches the maximum value before a gradual approach to a lower equilibrium value. This behaviour is due to the combination of two phenomena: water absorption and drug diffusion [24]. When water absorption predominates over drug diffusion, the microparticle diameter increases, reaching the maximum swollen value. On the contrary, when drug diffusion

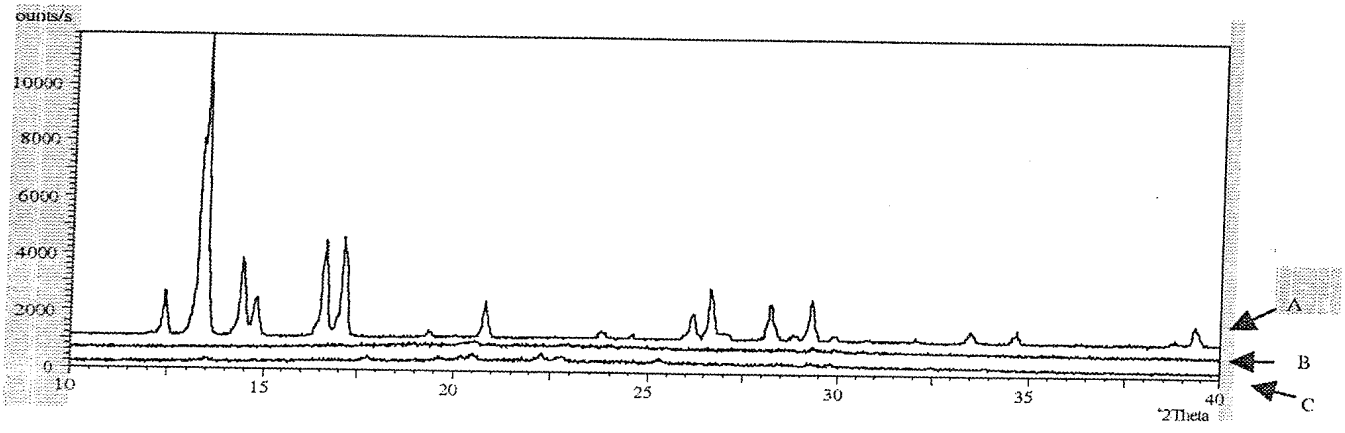

Fig. 6. X-ray diffraction patterns of pure diflunisal (A), drug unloaded PHG-MA (5.48) (B) and diflunisal-loaded PHG-MA (5.48) (C) samples. The number reported in brackets indicates the molar ratio between MA and PHG repeating unit.

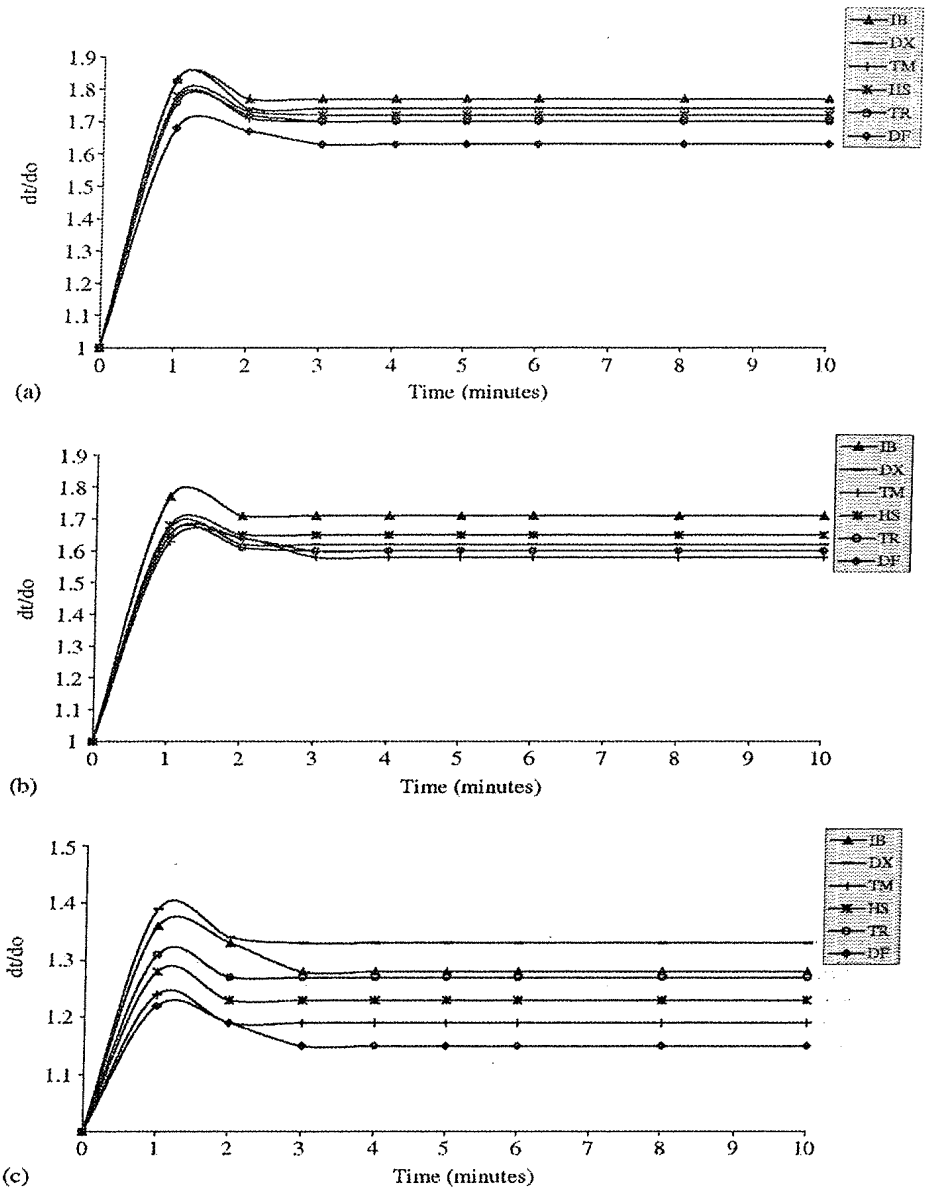

Fig. 7. Normalized diameter values (d_t/d_o) versus time for PHG-MA (5.48) microparticles containing trimethoprim (TM), dexamethasone (DX), histamine (HS), tyramine (TR), ibuprofen (IB) and diflunisal (DF) in: (a) doubly distilled water; (b) pH 6.8 phosphate buffer solution; (c) HCl 0.1 N solution. The number reported in brackets indicates the molar ratio between MA and PHG repeating unit.

prevails, the microparticle diameter decreases towards the lower equilibrium swollen value.

Anyway, drug-loaded microparticles reach an equilibrium swollen diameter lower than unloaded samples, probably because of interactions drug-network which reduce the water uptake. However, drug-loaded microparticles show a dynamic swelling behaviour analogous to unloaded samples, i.e. doubly distilled water > pH 6.8 > pH 1.0.

In order to evaluate the potential use of PHG-MA (5.48) micropartcles as pH-sensitive systems for oral drug delivery, in vitro release studies have been performed in simulated gastrointestinal fluids. The experiments have been carried out at 37°C at pH 1 (simulated gastric fluid) and pH 6.8 (simulated intestinal fluid) using the pH change method (see experimental section). Fig. 8 depicts drug release, expressed as the percent of drug (related to the entrapped total dose) delivered as a function of time, from PHG-MA (5.48) microparticles.

The experimental data reveal a release complete in a few minutes for trimethoprim, incomplete, within 2 h, for the other investigated drugs; for this reason, we have prolonged the experiment by changing pH from 1.0 to

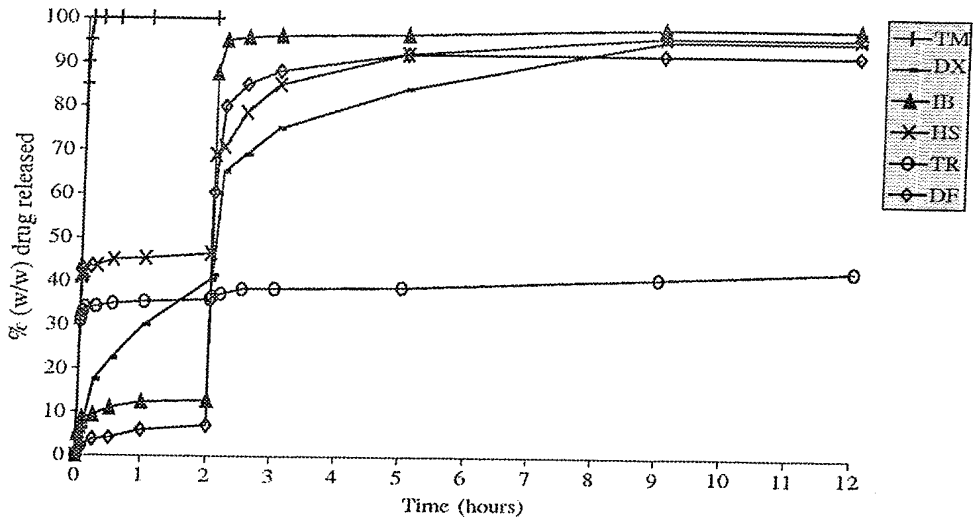

Fig. 8. Release of entrapped drugs from PHG-MA (5.48) sample at pH 1 from 0 to 2 h and at pH 6.8 from 2 to 24 h. The number reported in brackets indicates the molar ratio between MA and PHG repeating unit. The released drugs were: trimethoprim (TM), dexamethasone (DX), histamine (HS), tyramine (TR), ibuprofen (IB) and diflunisal (DF).

6.8 and the release was followed until 24 h. Drug release of diflunisal and ibuprofen is quite low at pH 1.0, whereas it increases quickly at pH 6.8. On the contrary, for tyramine drug release is rapid at pH 1.0, but it is not complete also after pH change. For dexamethasone and histamine about 40% of the drug is released within 2 h at pH 1 then the release increases with the pH change and it becomes complete within 10 h.

Apart from the trimethoprim, that is quickly released probably because it is preferably adsorbed on the surface of the microparticles and the tyramine whose release is not complete, for the other drugs it is possible to observe a remarkable variation in the amount of drug released when the pH changes from 1.0 to 6.8.

This effect is more marked in the case of drugs that contain acidic groups, such as ibuprofen and diflunisal, that are undissociated at acidic pH where only a low amount (< 10% w/w) is released. When the pH is 6.8, the dissociated form of ibuprofen and diflunisal prevails and the swelling of the network increases; both these factors cause a significant increase in the amount of released drug. On the contrary, for basic drugs, such as histamine and tyramine, an amount of about 45% and 35% w/w, respectively, is released within 2 h at pH 1, since in acidic medium, these molecules are in the ionized form. At pH 6.8 the amount of histamine in the ionized form is obviously lower than that found at pH 1, but its release is greater due to the increased swelling of the sample in this medium. A particular behaviour has been observed for tyramine-loaded microparticles, in fact, even though the pKa of tyramine is similar to that of histamine, the release of tyramine remains incomplete also after pH change. We suppose that the presence of phenolic group in the tyramine molecules could cause a great interaction with the polar groups of the polymeric network thus reducing the drug release. An analogous behaviour has been elsewhere reported for cytarabine (a polar drug) entrapped in other polyaminoacidic networks [25]. Finally for dexamethasone, that does not undergo ionization in aqueous medium, the different release between pH 1 and 6.8 is only imputable to the different swelling in these media.

We have also performed in vitro release studies for PHG (a) microparticles (that does not contain pendant acidic groups) loaded with some drugs. In particular, we have chosen ibuprofen as an example of acidic drug, dexamethasone as a neutral drug and histamine as a basic molecule (see Fig. 9).

It is evident that at pH 1.0, the PHG (a) microparticles release amounts of ibuprofen and dexamethasone greater than those released from PHG-MA (5.48) because PHG (a) at acidic pH undergoes a swelling greater that PHG-MA (5.48) as indicated by the lower Y value reported in Table 2 and by the dynamic swelling in Fig. 5. When pH is 6.8, a further increase of the drug release occurs also from PHG (a) microparticles. However, this increase is lower that that found for PHG-MA (5.48) microparticles in fact, the release is complete for ibuprofen only, wehereas the release of dexamethasone and histamine from the sample PHG (a) it is incomplete also after 24 h. Probably, for PHG (a) microparticles, the low increase in the swelling at pH 6.8 (due to the absence of pendent acidic groups) is not sufficient to promote the total release of entrapped drugs.

The obtained results evidence that PHG-MA (5.48) microparticles act as pH-sensitive hydrogels, since they show a swelling behaviour dependent on the pH of the

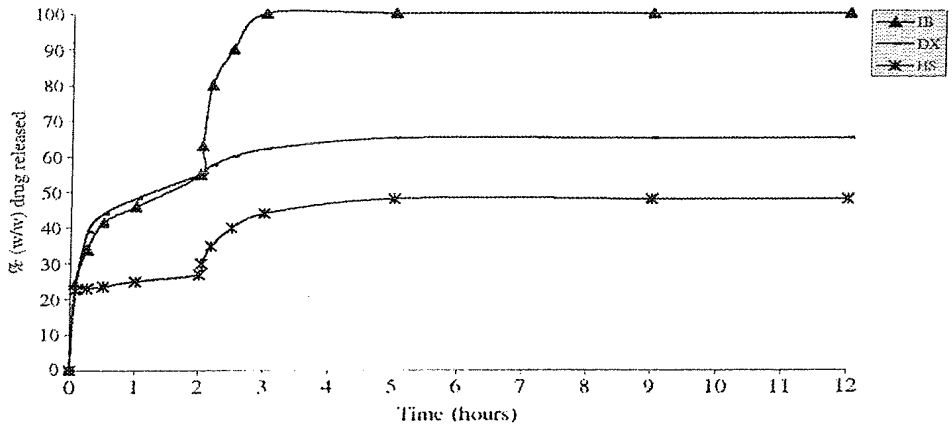

Fig. 9. Release of entrapped drugs from PHG (a) sample at pH 1 from 0 to 2 h and at pH 6.8 from 2 to 24 h. The released drugs were: dexamethasone (DX), histamine (HS) and ibuprofen (IB).

penetrating media. According to the release data, the sample PHG-MA (5.48) seems to be particularly suitable for the release of acidic drugs, for which a considerable variation in the amount of drug released has been observed when the pH changes from 1 to 6.8.

4. Conclusion

PHG, i.e. the copolymer obtained by partial derivatization of α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide (PHEA) with glycidylmethacrylate (GMA), has been crosslinked in the presence of acidic monomers, such as acrylic and methacrylic acid by using a reverse phase suspension polymerization method.

All obtained microparticles showed a spherical shape, a porous surface and a narrow size distribution. Swelling studies revealed a pH-dependent behaviour in media which simulate gastrointestinal fluids. The possibility to employ these microparticles as pH-sensitive systems has been evaluated by loading the sample that showed the greatest difference in the swelling between pH 1.0 and 6.8 with various drugs. In vitro release studies suggested the potential use of these microparticles for the release in the intestinal tract of acidic drugs, such as ibuprofen and diflunisal.

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